## THE CLAIMS

What is claimed is:

1. An independently functioning expression cassette comprising a nucleotide sequence encoding:

   (a) an origin of replication; and

   (b) a nucleotide sequence encoding a plasmid maintenance system comprising:

      (i) at least one post-segregational killing function; and

      (ii) at least one partitioning function.

2. The independently functioning expression cassette of claim 1 wherein the origin of replication is selected from the group consisting of: *ori*E1, *ori*101, *ori*15A and derivatives thereof.

3. The independently functioning expression cassette of claim 1 wherein the post-segregational killing function is selected from the group consisting of *asd, ssb, phd-doc* and *hok-sok.*

4. The independently functioning expression cassette of claim 1 wherein the post-segregational killing function is a substantial homologue of a naturally-occuring post-segregational killing function.

5. The independently functioning expression cassette of claim 1 wherein the partitioning function comprises an active partitioning function.

6. The independently functioning expression cassette of claim 1 wherein the partitioning function comprises a passive partitioning function.

7. The independently functioning expression cassette of claim 1 wherein the partitioning function is the *par* locus of pSC101.

8. The independently functioning expression cassette of claim 1 wherein the partitioning function comprises *parA.*

9. The independently functioning expression cassette of claim 1 wherein the partitioning function is a substantial homologue of a naturally-occurring partitioning function.

10. An amplifiable plasmid replicon comprising the independently functioning expression cassette of claim 1.

11. The amplifiable plasmid replicon of claim 10 further comprising independently functioning expression cassette comprising a nucleotide sequence encoding an antigen of interest transcriptionally controlled by a promoter.

12. The amplifiable plasmid replicon of claim 11 having a copy number that can be controlled to vary from 0 copies per cell to greater than 75 copies per cell and wherein the antigen of interest is a regulated test antigen for expression in a bacterium such that the inducible promoter is positioned to control expression of the nucleotide sequence, such that as induction of the promoter is increased, test antigen expression is increased, and the metabolic burden of the bacterium is increased.

13. A bacterial cell comprising the amplifiable plasmid replicon of claim 12.

14. The amplifiable plasmid replicon of claim 11 wherein the promoter is derived from the *ompC* promoter.

15. The amplifiable plasmid replicon of claim 11 wherein the promoter is the *ompC* promoter.

16. The amplifiable plasmid replicon of claim 11 wherein the promoter is the *ompC* promoter fragment from *E. coli* spanning nucleotides +70 through -389.

17. The amplifiable plasmid replicon of claim 11 wherein the promoter is a modified *ompC* promoter characterized in that said modified *ompC* promoter exhibits higher rates of osmotically regulated expression in relation to a corresponding *ompC* promoter without such point mutations.

18. The amplifiable plasmid replicon of claim 17 wherein the modified *ompC* promoter comprises a modified *Bgl*II site.

19. The amplifiable plasmid replicon of claim 17 wherein the modified *ompC* promoter is without a *Bgl*II site.

20. The amplifiable plasmid replicon of claim 18 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' beginning with the modified *Bgl*II site and ending with the ATG start codon:
AGATCX$^1$X$^2$TAAX$^3$CATCCACAGGAGGATATCTGATG, wherein:

(a) X$^1$ is selected from the group consisting of G, C and A;

(b) X$^2$ is an insert having from 1 to 5 nucleotides;

(c) $X^3$ is selected from the group consisting of A, T, G and C.

21. The amplifiable plasmid replicon of claim 18 wherein $X^1$ is G.

22. The amplifiable plasmid replicon of claim 18 wherein $X^2$ has from 1 to 4 nucleotides.

23. The amplifiable plasmid replicon of claim 18 wherein $X^2$ has 4 nucleotides.

24. The amplifiable plasmid replicon of claim 18 wherein $X^2$ has 4 nucleotides, independently selected from the group consisting of A, T and C.

25. The amplifiable plasmid replicon of claim 18 wherein $X^2$ comprises a nucleotide or nucleotide sequence selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

26. The amplifiable plasmid replicon of claim 18 wherein $X^2$ is selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

27. The amplifiable plasmid replicon of claim 18 wherein $X^2$ is ATCT.

28. The amplifiable plasmid replicon of claim 18 wherein $X^3$ is A.

29. The amplifiable plasmid replicon of claim 11 wherein the antigen of interest comprises the green fluorescent protein or a functional equivalent thereof.

30. The amplifiable plasmid replicon of claim 11 wherein the antigen of interest comprises a detoxified Shiga toxin and/or a substantial homologue thereof.

31. The amplifiable plasmid replicon of claim 11 wherein the antigen of interest comprises a Shiga toxin 2 antigen selected from the group comprising of: Shiga toxin 2 B subunit pentamers and genetically detoxified Shiga toxin 2 (Stx 2).

32. The amplifiable plasmid replicon of claim 30 wherein the gene encoding the detoxified Shiga toxin 2 has modified segments selected from the group consisting of:

(797) - ACA GCA GAC GCG TTA - (811);

(902) - CTG AAC CTA GGG CGA (916);

(1345) - GAA TTC GCG ACC AGT - (1359); and

(1435) - GAA TCA GAT TCT GGA - (1449).

33. A bacterial cell comprising the amplifiable plasmid replicon of claim 11.

34. The amplifiable plasmid replicon of claim 10 further comprising an independently functioning expression cassette comprising a nucleotide sequence encoding a selectable marker.

35. The amplifiable plasmid replicon of claim 34 wherein the selectable marker does not confer resistance to any antibiotic which is ordinarily used in medical treatment of humans.

36. The amplifiable plasmid replicon of claim 34 wherein the selectable marker comprises β-lactamase and/or a functional equivalent thereof.

37. The amplifiable plasmid replicon of claim 34 wherein the nucleotide sequence encoding the resistance marker is selected from the group consisting of *tetA*, *bla* and functional equivalents thereof.

38. A cell comprising the amplifiable plasmid replicon of claim 34.

39. A bacterial cell comprising the amplifiable plasmid replicon of claim 34.

40. An attenuated bacterial vector vaccine comprising a bacterial species containing a replicon, said replicon comprising:

(a) a nucleotide sequence encoding an antigen of interest; and

(b) a nucleotide sequence encoding a plasmid maintenance system.

41. The bacterial vector vaccine of claim 40 wherein the nucleotide sequence encoding the antigen of interest is contained in an independently functioning genetic cassette.

42. The bacterial vector vaccine of claim 40 wherein the nucleotide sequence encoding the plasmid maintenance system is contained within independently functioning genetic cassettes.

43. The attenuated bacterial live vector vaccine of claim 40 wherein the bacterial species is *Salmonella typhi*.

44. The attenuated bacterial live vector vaccine of claim 40 wherein the replicon further comprises an *ompC* promoter, or a substantial homologue thereof, controlling expression of the antigen of interest.

45. The attenuated bacterial live vector vaccine of claim 40 wherein the replicon further comprises a modified *ompC* promoter controlling expression of the antigen of interest, wherein the modified *ompC* promoter exhibits higher rates of osmotically controlled expression as compared to a non-modified *ompC* promoter.

46. The attenuated bacterial live vector vaccine of claim 45 wherein the modified *ompC* promoter comprises a modified *Bgl*II site.

47. The attenuated bacterial live vector vaccine of claim 45 wherein the modified *ompC* promoter is without a complete *Bgl*II site.

48. The attenuated bacterial live vector vaccine of claim 46 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' beginning with the modified *Bgl*II site and ending with the ATG start codon:
AGATCX$^1$X$^2$TAAX$^3$CATCCACAGGAGGATATCTGATG, wherein:

(a) X$^1$ is selected from the group consisting of G, C and A;

(b) X$^2$ is optionally present and is an insert having from 1 to 5 nucleotides;

(c) X$^3$ is selected from the group consisting of A, T, G and C.

49. The attenuated bacterial live vector vaccine of claim 48 wherein X$^1$ is G.

50. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ has from 1 to 4 nucleotides.

51. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ has 4 nucleotides.

52. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ has 4 nucleotides, independently selected from the group consisting of A, T and C.

53. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ comprises a nucleotide or nucleotide sequence selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

54. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ is selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

55. The attenuated bacterial live vector vaccine of claim 48 wherein X$^2$ is ATCT.

56. The attenuated bacterial live vector vaccine of claim 48 wherein X$^3$ is A.

57. The attenuated bacterial live vector vaccine of claim 40 wherein the plasmid maintenance system comprises:

(a) at least one post-segregational killing function; and

(b) at least one partitioning function.

58. The attenuated bacterial live vector vaccine of claim 57 wherein the post-segregational killing function is selected from the group consisting of balanced lethal functions, proteic functions and antisense functions.

59. The attenuated bacterial live vector vaccine of claim 57 wherein the post-segregational killing function is selected from the group consisting of *asd, ssb, phd-doc, hok-sok, and substantial homologues thereof.*

60. The attenuated bacterial live vector vaccine of claim 57 wherein the partitioning function comprises an active partitioning function.

61. The attenuated bacterial live vector vaccine of claim 57 wherein the partitioning function comprises a passive partitioning function.

62. The attenuated bacterial live vector vaccine of claim 57 wherein the partitioning function comprises the *par* locus of pSC101 and/or a substantial homologue thereof.

63. The attenuated bacterial live vector vaccine of claim 57 wherein the partitioning function comprises *parA* and/or a substantial homologue thereof.

64. The attenuated bacterial live vector vaccine of claim 40 wherein the antigen of interest is a test antigen.

65. The attenuated bacterial live vector vaccine of claim 64 wherein the test antigen is selected from the group consisting of green fluorescent protein, functional equivalents of green fluorescent protein and substantial homologues of green fluorescent protein.

66. The attenuated bacterial live vector vaccine of claim 40 wherein the antigen of interest is a detoxified Shiga toxin.

67. The attenuated bacterial live vector vaccine of claim 40 wherein the antigen is one or more Shiga toxin 2 antigens selected from the group comprising Shiga toxin 2 B subunit pentamers and a genetically detoxified Stx 2.

68. The attenuated bacterial live vector vaccine of claim 67 wherein the gene encoding the detoxified Shiga toxin 2 has mutations selected from the group consisting of:

(797) - ACA GCA GAC GCG TTA - (811);

(902) - CTG AAC CTA GGG CGA - (916);

(1345) - GAA TTC GCG ACC AGT - (1359); and

(1435) - GAA TCA GAT TCT GGA - (1449).

69. The attenuated bacterial live vector vaccine of claim 40 wherein the replicon further comprises a nucleotide sequence encoding a resistance marker.

70. The attenuated bacterial live vector vaccine of claim 69 wherein the nucleotide sequence encoding the resistance marker is contained in an independently functioning genetic cassette.

71. The attenuated bacterial live vector vaccine of claim 69 wherein the selectable marker does not confer resistance to any antibiotic which is ordinarily used in medical treatment of humans

72. The attenuated bacterial live vector vaccine of claim 69 wherein the nucleotide sequence encoding the resistance marker comprises *bla* and/or a substantial homologue thereof.

73. The attenuated bacterial live vector vaccine of claim 69 wherein the nucleotide sequence encoding the resistance marker comprises *tetA* and/or a substantial homologue thereof.

74. A conditionally unstable plasmid for examining changes in plasmid stability resulting from incorporation of plasmid maintenance systems, said plasmid comprising an origin of replication yielding an average copy number which falls within the range of from about 2 to about 75 copies and a promoter driving the expression of a protein or peptide, overexpression of which imposes a metabolic burden on a bacterium, which favors plasmid loss.

75. The conditionally unstable plasmid of claim 74 wherein the average copy number falls within the range of about 5 to about 60 copies.

76. The conditionally unstable plasmid of claim 74 wherein the promoter comprised an *ompC* promoter or a substantial homologue thereof.

77. The conditionally unstable plasmid of claim 74 wherein the protein or peptide is selected from the group consisting of green fluorescent protein, functional equivalents of green fluorescent protein and substantial homologues of green fluorescent protein

78. The conditionally unstable plasmid of claim 74, wherein the average copy number is selected from the group consisting of: about 5 copies per cell; about 15 copies per cell; and about 60 copies per cell.

79. The conditionally unstable plasmid of claim 74 wherein the origin of replication is selected from the group consisting of the origin of replication of plasmid pSC101, origin of replication of plasmid pACYC184, origin of replication of plasmid pAT153, and substantial homologues of any of such origins of replication.

80. The conditionally unstable plasmid of claim 74 wherein the origin of replication is from pSC101, conferring a copy number of approximately 5 copies per genome equivalent.

81. The conditionally unstable plasmid of claim 74 wherein the origin of replication is from pACYC184, conferring a copy number of approximately 15 copies per genome equivalent.

82. The conditionally unstable plasmid of claim 74 wherein the origin of replication is from pAT153, conferring a copy number of approximately 60 copies per genome equivalent.

83. A method for eliciting an immune response in a subject comprising administering to the subject a bacterial live vector vaccine comprising a bacterial strain comprising an expression vector comprising:

(a) a nucleotide sequence encoding an antigen;

(b) a promoter controlling expression of the antigen; and

(c) a nucleotide sequence encoding at least one plasmid maintenance system.

84. The method of claim 83 wherein the bacterial live vector vaccine is administered in an immunizingly effective amount.

85. The method of claim 83 wherein the bacterial live vector is an attenuated *Salmonella typhi* species.

86. The method of claim 83 wherein (a) and (b) are contained in an independently functioning genetic cassette.

87. The method of claim 83 wherein (c) is contained within independently functioning genetic cassettes.

88. The method of claim 83 wherein the promoter is an inducible promoter.

89. The method of claim 83 wherein the promoter is an *ompC* promoter or a functional equivalent thereof.

90. The method of claim 83 wherein the promoter is a modified *ompC* promoter, phenotypically characterized in that said promoter exhibits higher rates of osmotically controlled expression in relationship to a corresponding *ompC* promoter without such point mutations.

91. The method of claim 90 wherein the modified *ompC* promoter comprises a modified *Bgl*II site, or is without a complete *Bgl*II site.

92. The method of claim 91 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' beginning with the modified *Bgl*II site and ending with the ATG start codon: AGATCX$^1$X$^2$TAAX$^3$CATCCACAGGAGGATATCTGATG, wherein:

(a) X$^1$ is selected from the group consisting of G, C and A;

(b) X$^2$ is an insert having from 1 to 5 nucleotides;

(c) X$^3$ is selected from the group consisting of A, T, G and C.

93. The method of claim 92 wherein X$^1$ is G.

94. The method of claim 92 wherein X$^2$ has from 1 to 4 nucleotides.

95. The method of claim 92 wherein X$^2$ has 4 nucleotides.

96. The method of claim 92 wherein X$^2$ has 4 nucleotides, independently selected from the group consisting of A, T and C.

97. The method of claim 92 wherein X$^2$ comprises a nucleotide or nucleotide sequence selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

98. The method of claim 92 wherein X$^2$ is selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

99. The method of claim 92 wherein X$^2$ is ATCT.

100. The method of claim 92 wherein X$^3$ is A.

101. The method of claim 83 wherein the plasmid maintenance system comprises:

(a) at least one post-segregational killing function; and

(b) at least one partitioning plasmid function.

102. The method of claim 101 wherein the post-segregational killing function is contained in an independently functioning genetic cassette.

103. The method of claim 101 wherein the post-segregational killing function is selected from the group consisting of balanced lethal functions, proteic functions and antisense functions.

104. The method of claim 101 wherein the post-segregational killing function is selected from the group consisting of *asd, ssb, phd-doc*, *hok-sok* and substantial homologues thereof.

105. The method of claim 101 wherein the partitioning function is contained in an independently functioning genetic cassette.

106. The method of claim 101 wherein the partitioning function comprises an active partitioning function.

107. The method of claim 101 wherein the partitioning function comprises a passive partitioning function.

108. The method of claim 101 wherein the partitioning function comprises the *par* locus of pSC101 and/or a substantial homologue thereof.

109. The method of claim 101 wherein the partitioning function comprises *parA* and/or a substantial homologue thereof.

110. The method of claim 101 wherein the antigen(s) comprise at least one genetically detoxified Shiga toxin.

111. The method of claim 101 wherein the antigen(s) include at least one Shiga toxin 2 (Stx2) antigen selected from the group comprising Shiga toxin 2 B subunit pentamers and a genetically detoxified Stx 2.

112. The method of claim 83 wherein the nucleotide sequence further comprises a nucleotide sequence encoding a selectable marker which does not confer resistance to any antibiotic which is ordinarily used in the treatment of humans.

113. The method of claim 112 wherein the nucleotide sequence encoding the selectable marker is contained in an independently functioning genetic cassette.

114. The method of claim 83 wherein the subject is a human.

115. The method of claim 83 wherein the subject is a bovine.

116. A method of making a stabilized bacterial live vector vaccine comprising transforming a bacterial live vector with a replicon comprising:

(a) a plasmid maintenance system comprising:

(i) at least one post-segregational killing function; and

(ii) at least one partitioning function; and

(b) a nucleotide sequence encoding one or more antigens.

117. The method of claim 116 wherein the post segregation killing function is contained in an independently functioning genetic cassette.

118. The method of claim 116 wherein the partitioning function is contained in an independently functioning genetic cassette.

119. The method of claim 116 wherein the post-segregational killing function is selected from the group consisting of balanced lethal functions, proteic functions and antisense functions.

120. The method of claim 116 wherein the post-segregational killing function is selected from the group consisting of *asd, ssb, phd-doc*, *hok-sok*, and substantial homologues thereof.

121. The method of claim 116 wherein the partitioning function is an active partitioning function.

122. The method of claim 116 wherein the partitioning function is a passive partitioning function

123. The method of claim 116 wherein the partitioning function comprises the *par* locus of pSC101 and/or a substantial homologue thereof.

124. The method of claim 116 wherein the partitioning function comprises *parA* and/or a substantial homologue thereof.

125. The method of claim 116 wherein the one or more antigens comprise at least one detoxified Shiga toxin.

126. The method of claim 116 wherein the one or more antigens comprise one or more Shiga toxin 2 antigens selected from the group comprising Shiga toxin 2 B subunit pentamers and a detoxified Stx 2.

127. The method of claim 125 wherein the gene encoding the detoxified Shiga toxin 2 has mutations selected from the group consisting of:

(797) - ACA GCA GAC GCG TTA - (811);

(902) - CTG AAC CTA GGG CGA - (916);

(1345) - GAA TTC GCG ACC AGT - (1359); and

(1435) - GAA TCA GAT TCT GGA - (1449).

128. The method of claim 116 wherein the promoter is a modified *ompC* promoter phenotypically characterized in that said promoter exhibits higher rates of osmotically regulated expression in relation to a corresponding *ompC* promoter without such point mutations.

129. The method of claim 116 wherein the modified *ompC* promoter is without a *Bgl*II site.

130. The method of claim 116 wherein the modified *ompC* promoter comprises a mutated *Bgl*II site.

131. The method of claim 116 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' beginning with the mutated *Bgl*II site and ending with the ATG start codon: AGATCX$^1$X$^2$TAAX$^3$CATCCACAGGAGGATATCTGATG, wherein:

(a) $X^1$ is selected from the group consisting of G, C and A;

(b) $X^2$ is an insert having from 1 to 5 nucleotides;

(c) $X^3$ is selected from the group consisting of A, T, G and C.

132. A DNA comprising a modifed *ompC* promoter, phenotypically characterized in that said promoter exhibits higher rates of osmotically regulated expression in relation to a corresponding non-mutated *ompC* promoter.

133. The DNA of claim 132 wherein the modified *ompC* promoter comprises a mutated *Bgl*II site.

134. The DNA of claim 133 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' beginning with the mutated *Bgl*II site and ending with the ATG start codon: AGATCX$^1$X$^2$TAAX$^3$CATCCACAGGAGGATATCTGATG, wherein:

(a) $X^1$ is selected from the group consisting of G, C and A;

(b) $X^2$ is an insert having from 1 to 5 nucleotides;

(c) $X^3$ is selected from the group consisting of A, T, G and C.

135. The DNA of claim 134 wherein $X^1$ is G.

136. The DNA of claim 134 wherein $X^2$ has from 1 to 4 nucleotides.

137. The DNA of claim 134 wherein $X^2$ has 4 nucleotides.

138. The DNA of claim 134 wherein $X^2$ has 4 nucleotides, independently selected from the group consisting of A, T and C.

139. The DNA of claim 134 wherein $X^2$ comprises a nucleotide or nucleotide sequence selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

140. The DNA of claim 134 wherein $X^2$ is selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

141. The DNA of claim 134 wherein $X^2$ is ATCT.

142. The DNA of claim 134 wherein $X^3$ is A.

143. The DNA of claim 133 wherein the mutated *Bgl*II site of the *omp*C promoter comprises the sequence: AGATCG.

144. The DNA of claim 133 wherein the mutated *Bgl*II site of the *omp*C promoter consists of the sequence: AGATCG.

145. The DNA of claim 133 wherein the modified *ompC* promoter comprises the following sequence 5' to 3' between the mutated *Bgl*II site and the ATG start codon: AGATCTTAAACATCCACAGGAGGATATCTGATG.

146. The DNA of claim 132 further comprising a plasmid maintenance system comprising:

(a) at least one post-segregational killing function; and

(b) at least one partitioning function.

147. An expression plasmid comprising the DNA of claim 146.

148. The DNA of claim 132 further comprising a nucleotide sequence encoding a peptide or protein, the expression of which is controlled by said modified promoter.

149. The DNA of claim 147, wherein the peptide or protein is selected from the group consisting of: heterologous antigens and green fluorescent protein.

150. The DNA of claim 147, wherein the peptide or protein is selected from the group consisting of: detoxified Shiga toxins.

151. The DNA of claim 147, wherein the peptide or protein is selected from the group consisting of: Shiga toxin 2 B subunit pentamers and a detoxified Stx 2.

152. The DNA of claim 132 further comprising a nucleotide sequence encoding a selectable marker, which marker does not confer resistance to any antibiotic which is ordinarily used in the treatment of humans.

153. An expression plasmid comprising the DNA of claim 152.

154. The DNA of claim 132 further comprising an origin of replication and a transcription terminator sequence in a 5' position in relation to the origin of replication such that transcription of the origin of replication is less perturbed relative to perturbance in the absence of such transcriptional terminator sequence.

155. An expression plasmid comprising the DNA of any of claim 132.